Determination and analysis of the genome sequence of *Spodoptera littoralis* multiple nucleopolyhedrovirus

Jonathan E. Breitenbach [a], El-Sayed A. El-Sheikh [a,1], Robert L. Harrison [b], Daniel L. Rowley [b], Michael E. Sparks [b], Dawn E. Gundersen-Rindal [b], Holly J.R. Popham [a,*]

[a] *Biological Control of Insects Research Laboratory, USDA Agricultural Research Service, Columbia, MO, USA*
[b] *Invasive Insect Biocontrol and Behavior Laboratory, Plant Sciences Institute, USDA Agricultural Research Service, Beltsville, MD, USA*

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ABSTRACT

The *Spodoptera littoralis* multiple nucleopolyhedrovirus (SpliMNPV), a pathogen of the Egyptian cotton leaf worm *S. littoralis*, was subjected to sequencing of its entire DNA genome and bioassay analysis comparing its virulence to that of other baculoviruses. The annotated SpliMNPV genome of 137,998 bp was found to harbor 132 open reading frames and 15 homologous repeat regions. Four unique genes not present in SpltMNPV were identified, as were 14 genes that were absent or translocated by comparison. Bioassay analysis of experimentally infected *Spodoptera frugiperda* revealed an extended killing time for SpliMNPV as compared to *S. frugiperda* MNPV (SfMNPV), but a level of mortality similar to that caused by infection with SfMNPV and superior to that of *Autographa californica* MNPV (AcMNPV). Although extensive similarity was observed between the genome structure and predicted translation products of SpliMNPV and *Spodoptera litura* MNPV (SpltMNPV), genetic distances between isolates of SpliMNPV and SpltMNPV suggest that they are in fact different species of genus *Alphabaculovirus*.

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1. Introduction

Baculoviruses are large, double-stranded DNA viruses, grouped in family *Baculoviridae*, that cause lethal infection in arthropods and have been used as biological insecticides, for recombinant protein expression, and recently, as mammalian gene transduction vectors (Bonning and Nusawardani, 2007; Grabherr and Ernst, 2010; Hitchman et al., 2011; van Beek and Davis, 2007). Most baculoviruses typically feature a narrow host range both *in vivo* and *in vitro*, proving unable to replicate or cause symptomatic infections when introduced into alternate insect species or cells, often within the same genus (Thiem, 1997). Most known baculoviruses are nucleopolyhedroviruses (NPVs; genus *Alphabaculovirus*) or granuloviruses (GVs; genus *Betabaculovirus*) that infect lepidopteran species (Herniou et al., 2011). During infection of larvae, occlusion bodies (OBs) made up of enveloped virions encased in a crystalline matrix of polyhedrin protein are ingested by the host. Virions are liberated by dissolution of the OBs in the alkaline insect midgut, establishing a primary infection in proximal cells of the midgut. Replication of the virus in these cells produces enveloped, budded virus (BV) which subsequently disseminates the infection throughout the host. Late during infection, expression of viral polyhedrin results in the incorporation of viral particles into OBs, which are then released into the environment following virus-mediated liquefaction of the host (Miller, 1997).

Spodoptera spp. is a genus of generalist crop pests found throughout the Americas, Southeast Asia and countries around the Mediterranean (Ellis, 2004; Meagher et al., 2008). *Spodoptera littoralis*, the Egyptian cotton leafworm, is found in Africa, southern Europe, and the Middle East, where it is a particularly destructive pest of cotton and a wide range of economically important vegetables and ornamentals (Ellis, 2004). The emergence of resistance to the chemical insecticides used to control *S. littoralis* has prompted the development of alternative control measures (Horowitz et al., 1998; Jones et al., 1994). *S. littoralis* multiple nucleopolyhedrovirus (SpliMNPV) is an alphabaculovirus that has been isolated from populations of *S. littoralis* in Egypt, Morocco, France, Israel, the Azores islands, Tunisia, and Turkey (Cherry and Summers, 1985; Croizier et al., 1989; Kislev and Edelman, 1982; Laarif et al., 2011; Martins et al., 2005; Toprak and Gurkan, 2004). The host range of the virus has been shown to be narrow, likely infecting only within *Spodoptera* spp. (Takatsuka et al., 2007; Toprak et al., 2006). Extensive research, including partial sequencing, restriction enzyme

digestion pattern analysis, and growth characteristics under varied conditions have been carried out to describe the properties and insecticidal activity of SpliMNPV in the laboratory and field, as well as testing different formulations of the virus and effects on non-target species (Kunjeku et al., 1998; Laarif et al., 2011; Seufi, 2008; Toprak et al., 2006, 2007). Commercially available formulations of SpliMNPV for control of *S. littoralis* have been developed (El-Husseini, 2006; Ravensberg, 2011).

Early restriction endonuclease mapping studies revealed that SpliMNPV samples isolated from populations in Israel occurred in two genetically distinct classes, termed SpliMNPV-A (or -T) and SpliMNPV-B (or -D) (Cherry and Summers, 1985; Kislev and Edelman, 1982). Only SpliMNPV-B occurs in virus samples from other countries (Croizier et al., 1986; Martins et al., 2005). NPVs with restriction endonuclease fragment patterns matching those of SpliMNPV-B have been isolated from diseased *S. litura* larvae in Japan (Takatsuka et al., 2003). Partial nucleotide sequences from individual isolates of SpliMNPV-B have been published, including portions of the genomes of isolates SpliMNPV-M2 from Morocco (Croizier et al., 1989) and SpliMNPV-E15 from Egypt (Faktor et al., 1995). In this paper, we present the complete annotated genomic sequence of an Egyptian isolate of SpliMNPV-B and compare conserved features to baculoviruses isolated from other fully sequenced *Spodoptera* species and the prototypic baculovirus, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). We also report on the lethality of this isolate, SpliMNPV-AN1956, in neonatal *S. frugiperda* larvae and compare its biological activity to that of *S. frugiperda* MNPV (SfMNPV, strain SfMNPV-3) and AcMNPV (strain L1) in tandem bioassays.

2. Materials and methods

2.1. Viruses, insects, and infections

SpliMNPV-AN1956 was originally isolated by Abul Nasr in 1956 and an extensive study of its growth characteristics is available elsewhere (Abul Nasr, 1956; Grzywacz et al., 1998). *S. frugiperda* eggs and artificial diet (#9772) were commercially obtained from Bio-Serv (Frenchtown, NJ). Larvae were reared in a fashion consistent with previous studies (Harrison et al., 2008).

2.2. DNA isolation and sequencing

Isolation of polyhedra from insect cadavers and subsequent extraction of nucleic acid from OBs has been described (Harrison and Popham, 2008; Harrison et al., 2008). Briefly, 4th instar *S. frugiperda* larvae were fed a mixture of water, sucrose, blue food coloring, and viral OBs. Cadavers were collected in a 50 mL conical tube and approximately 20 mL of cadaver slurry was suspended in 20 mL of PBS and filtered twice through cheesecloth. Half was loaded onto a 40–60% sucrose gradient and centrifuged at 27,000 rpm for 1.5 h to obtain purified OBs which were then aspirated, washed, pelleted, resuspended in ddH$_2$O, and counted. To obtain genomic DNA, the remaining OBs were incubated in 15 mL of 100 mM sodium bicarbonate for 15 min and the undissolved OBs were pelleted by centrifugation at 3000 rpm. Undissolved OBs were subjected to a second treatment in 15 mL of 100 mM sodium bicarbonate and a final centrifugation step removed non-dissolved particles. The two 15 mL aliquots of enveloped virus were underplayed with a sucrose cushion and centrifuged at 27,000 rpm. The pellet containing virus particles was resuspended in ddH$_2$O and incubated at 37 °C in the presence of proteinase K (0.75 mg/mL final concentration) to release viral DNA from particles, and genomic DNA was subsequently phenol:chloroform extracted and resuspended in dH$_2$O following standard protocol (O'Reilly et al., 1993).

Ethanol-precipitated SpliMNPV DNA was pelleted by microcentrifugation and resuspended in distilled deionized H$_2$O. After resuspension, DNA was sheared, size fractionated, and a multiplexed Roche GS FLX Titanium library was prepared for sequencing at the Georgia Genomics Facility (https://gsle.ovpr.uga.edu). Initial sequencing was carried out on an in-house Roche 454 Jr instrument. Reads were sorted by multiplex identifier (MID) tags (SpliMNPV reads were MID tagged with the sequence ACACGACGACT) and *de novo* assembled using Roche's GSAssembler program with default parameters. Three contigs having lengths 104,680 bp, 32,408 bp and 648 bp resulted. The Consed sequence editing utility (Gordon et al., 1998) was used to perform manual adjustments to the assembly; gaps were closed, and a variety of sequencing ambiguities resolved, by PCR amplifying the corresponding genomic regions from viral DNA. PCR amplicon sequencing was performed on an ABI 3130xl Genetic Analyzer instrument using previously described procedures (Harrison and Lynn, 2007). The Lasergene SeqMan NGEN V3.0 (DNAStar) assembler and the SeqManPro V9 sequence editor were used to prepare the finalized contig, which was 137,998 bp in length with an average coverage of 157.09X.

Partial sequence data of the *polh*, *lef-8*, and *lef-9* genes of other SpliMNPV isolates were generated from SpliMNPV OB samples in a USDA Agricultural Research Service insect virus collection. OBs were solubilized as previously described (Rowley et al., 2010), and solubilized material as used as templates for PCR with a set of degenerate primers described by Lange et al. (Harrison and Lynn, 2007; Lange et al., 2004). PCR products were fully sequenced with M13 forward and reverse primers as previously described (Harrison and Lynn, 2007). For some isolates, custom primers were designed for amplification and sequencing of the three loci. Sequence data were assembled into contigs in DNASTAR Lasergene 9 (DNAStar, Madison, WI).

2.3. Genome sequence analysis

ORF identification and construction of the mapped genome was carried out using DNASTAR Lasergene 9 Core Suite software. ORFs that were at least 50 codons in length that did not overlap other ORFs by more than 75 nucleotides and were located outside of homologous repeat (*hr*) regions were selected for further analysis. Sequence similarity of individually translated ORFs to their counterparts, where present in other *Spodoptera* spp. viruses, including SpltMNPV (G2 strain) (GenBank ID: AF325155.1), SeMNPV (GenBank ID: NC_002169.1), SfMNPV (GenBank ID: NC_009011.2), and the prototypic baculovirus AcMNPV (C6 strain) (GenBank ID: L22858.1), was determined at the amino acid sequence level using NCBI blastp with standard settings. Homologous repeat regions were identified using the Tandem Repeats Finder program (Benson, 1999) and by visual examination of intergenic regions in the genome sequence.

Gene parity plots were constructed according to the method of Hu et al. (1998). For SpliMNPV, ORFs were numbered in ascending order beginning with the polyhedrin gene and corresponding ORFs in SpltMNPV, AcMNPV, SeMNPV, and SfMNPV were entered in parallel. Data sets are presented as a line graph in which each point represents an ORF. Deviations from a slope of 1 are indicative of the direction, distance, gaps, presence, and linearity of the ORFs between SpliMNPV and each of the other viruses.

2.4. Phylogeny

Partial sequences from the *polh*, *lef-8*, and *lef-9* genes of group II alphabaculoviruses and a granulovirus were aligned in DNASTAR Lasergene 10 using CLUSTAL W with default parameters (Thompson et al., 1994). Alignments were concatenated with BioEdit (Hall, 1999) and phylogenetic trees inferred from
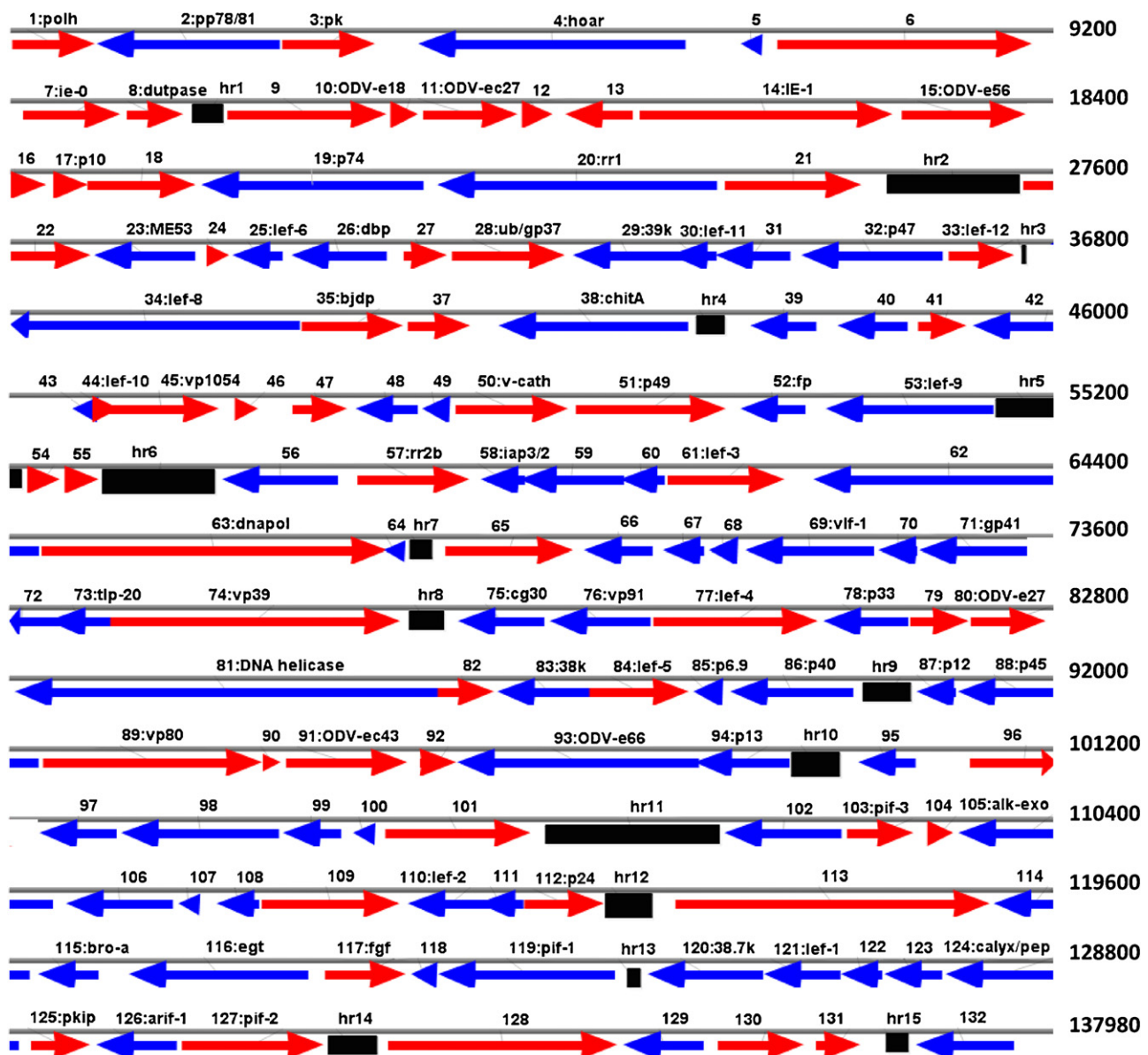
Fig. 1. Diagrammatical representation of the SpliMNPV genome. ORFs reading left to right are shown in blue arrows; ORFs reading right to left are shown in red. Darkened boxes denote an *hr* region, and numbers on the right indicate genome position. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the concatenated alignment using MEGA 5.05 (Tamura et al., 2011). Minimum evolution (ME) and maximum parsimony (MP) trees were generated using a close-neighbor-interchange heuristic search, starting with either one initial neighbor-joining tree (ME) or 10 initial trees generated by random addition of sequences (MP). For ME trees, Kimura 2-parameter distances were determined with a maximum likelihood-estimated gamma shape parameter of 0.4451. Tree reliability was tested with bootstrap re-sampling using 500 replicates.

2.5. Bioassay analysis of viruses

Neonate *S. frugiperda* larvae were infected *per os* by the droplet feeding method developed by Hughes and coworkers (Hughes et al., 1986) with six doses of occlusion bodies (OBs) ranging from 1×10^3 to 1×10^8 OBs/ml. Larvae were placed on fresh food, maintained at $28 \pm 1\,^{\circ}$C at a photoperiod of 14:10 h (L:D), and monitored two or three times daily for 7 days. The LC_{50} (concentration of OBs required to kill 50% of the test larvae) for each virus was calculated by Proc Logisitic using SAS vers. 9.1, as were hypotheses concerning the parallelism and equality of probit dose–response lines. Median mortality times (LT_{50}) were calculated with survivors excluded using the Kaplan–Meier Estimator and mortality data of the 1×10^7 OBs/mL dose for all the viruses. Comparison of LT_{50}s was computed using the log-rank test by SigmaPlot version 11 (Systat Software, Inc., San Jose, CA). The L1 strain of *A. californica* MNPV (AcMNPV) was also bioassayed for comparison against SpliMNPV and SfMNPV (Lee and Miller, 1979). All bioassays were repeated three times.

2.6. Nucleotide sequence accession numbers

The SpliMNPV-AN1956 nucleotide sequence is available from GenBank under accession number JX454574. The nucleotide sequences for additional SpliMNPV isolate lef-8, polh, and

Table 1
Features of the SpliMNPV–AN1956 genome.

ORF or feature	Name[a]	Position[b]	# of aa (size, Da)	Promoter motifs[c]	SpltMNPV ORF (aa) or hr	% ID (range)	SfMNPV ORF (aa)	% ID (range)	SeMNPV ORF (aa)	% ID (range)	AcMNPV ORF (aa)	% ID (range)
					Comparison with other NPVs							
1	polh	1 → 747	248 (29,089)	L	splt1 (249)	99 (248–249)	sf1 (246)	83 (207–248)	se1 (246)	84 (209–248)	ac8 (245)	85 (206–242)
2	pp 78/83	744 ← 2378	544 (60,605)	e	splt2 (548)	71 (403–565)	sf2 (460)	26 (93–360)	se2 (462)	21 (35–168)	ac9 (543)	24 (39–164)
3	pk-1	2380 → 3216	278 (32,597)		splt3 (270)	87 (237–272)	sf3 (278)	44 (110–251)	se3 (295)	48 (120–251)	ac10 (272)	44 (109–246)
4	hoar	3578 ← 5956	792 (89,081)	EC	splt4 (731)	64 (515–800)	sf4 (470)	23 (54–240)	se4 (724)	25 (59–238)		
5		6420 ← 6614	64 (7268)									
6		6742 → 9006	754 (85,500)		splt7 (717)	60 (398–665)						
7	le-0	9297 → 10,172	291 (33,483)	L	splt8 (289)	89 (259–290)			se138 (92)		ac141 (261)	32 (75–232)
8	dutpase	10,211 → 10,726	171 (18,699)		splt10 (164)	80 (116–131)	sf54 (144)	44 (60–137)	se55 (143)	45 (62–137)		
hr1	(5 P-I repeats)	10,794...11,070			hr1 (4 P-I repeats)							
9		11,097 → 12,518	473 (56,043)	L	splt11 (469)	97 (459–473)			se137 (460)	50 (230–464)	ac142 (477)	45 (213–475)
10	odv-e18	12,544 → 12,795	83 (9222)	e, L	splt12 (83)	100 (83–83)	sf140 (81)	79 (19–24)	se136 (80)	61 (41–67)	ac143 (62)	71 (17–24)
11	odv-ec27	12,819 → 13,673	284 (56,043)	EC, L	splt13 (283)	97 (276–284)	sf139 (283)	53 (141–268)	se135 (281)	49 (140–283)	ac144 (290)	46 (131–284)
12		13,701 → 13,982	93 (10,793)	L	splt14 (93)	94 (87–93)	sf138 (92)	54 (50–93)	se134 (92)	53 (49–93)	ac145 (77)	44 (34–77)
13		14,073 ← 14,687	204 (22,509)	–	splt15 (200)	80 (165–206)	sf137 (111)	30 (13–43)	se133 (200)	32 (67–208)	ac146 (201)	30 (62–208)
14	i.e.-1	14,729 → 16,978	749 (85,128)	EC	splt16 (688)	73 (508–697)	sf136 (682)	32 (137–427)	se132 (714)	33 (142–430)	ac147 (582)	28 (109–390)
15	odv-e56	17,040 → 18,152	370 (40,213)	L	splt17 (371)	93 (328–351)	sf9 (160)	53 (169–320)	se6 (371)	46 (159–346)	ac148 (376)	49 (168–343)
16		18,167 → 18,718	183 (20,938)	e, L	splt18 (183)	95 (174–183)	sf125 (178)	50 (88–176)	se124 (187)	56 (93–166)	ac34 (215)	34 (51–152)
17	p10	18,771 → 19,085	104 (11,138)	e, L	splt19 (105)	95 (100–105)	sf132 (105)	50 (45–90)	se130 (88)	46 (40–87)	ac137 (94)	40 (22–55)
18		19,063 → 20,034	323 (37,254)	–	splt20 (314)	81 (261–324)						
19	p74	20,068 ← 22,044	658 (75,724)	EC, L	splt21 (657)	93 (614–661)	sf134 (646)	48 (315–646)	se131 (653)	51 (334–657)	ac138 (645)	56 (327–589)
20	rr1	22,146 → 24,632	828 (93,046)		splt23 (770)	87 (681–785)			se139 (770)	52 (393–762)		
21		24,682 → 25,902	406 (48,382)		splt24 (399)	59 (240–410)						
hr2	(10 P-I and 6 P-II-like repeats)	26,117...27,290			hr2 and hr3 (6 P-I and 4 P-II repeats)							
22		27,305 → 28,309	334 (38,818)		splt26 (297)	90 (273–303)						
23	me53	28,322 ← 29,236	304 (36,542)	L	splt27 (301)	94 (283–301)	sf10 (363)	24 (78–326)	se7 (390)	26 (84–328)	ac139 (449)	22 (66–299)
24		29,324 → 29,530	68 (8113)		splt28 (86)	76 (52–68)	sf130 (94)	41 (24–58)	se128 (136)	41 (28–68)	ac29 (71)	36 (21–59)
25	lef-6	29,541 → 30,002	153 (18,132)	L	splt29 (145)	70 (111–159)	sf128 (154)	26 (20–78)	se127 (163)	27 (19–70)	ac28 (173)	34 (13–38)
26	dbp	30,061 → 30,921	286 (32,642)	e	splt30 (290)	81 (234–290)	sf127 (331)	30 (77–255)	se126 (328)	30 (79–260)	ac25 (316)	24 (61–252)
27		31,049 → 31,450	133 (15,185)		splt31 (149)	90 (120–149)	sf126 (133)	40 (41–103)	se125 (135)	33 (8–24)	ac26 (129)	37 (43–115)
28	ubi/gp37	31,476 → 32,489	337 (38,038)		splt32 (351)	92 (310–337)	sf24 (260)	51 (130–257)	se25 (267)	56 (126–243)	ac64 (302)	53 (126–239)
29	39k	32,541 ← 33,533	330 (37,243)	e	splt33 (322)	78 (264–340)	sf121 (310)	32 (52–165)	se120 (317)	34 (55–164)	ac36 (275)	30 (79–260)
30	lef-11	33,397 → 33,828	143 (16,751)		splt34 (144)	85 (123–144)	sf120 (133)	39 (36–93)	se119 (103)	41 (39–94)	ac37 (112)	33 (31–93)
31		33,798 → 34,478	226 (27,068)	EC, e, L	splt35 (220)	88 (199–226)	sf119 (244)	51 (110–217)	se118 (261)	50 (115–231)	ac38 (216)	45 (98–216)
32	p47	34,555 → 35,820	843 (48,947)		splt36 (422)	100 (422–422)	sf116 (399)	56 (234–418)	se115 (400)	56 (235–418)	ac40 (401)	49 (204–418)
33	lef-12	35,853 → 36,449	198 (23,013)		splt37 (201)	85 (168–198)					ac41 (181)	30 (32–108)
hr3	(1 P-1 repeat)	36,506...36,547			hr4 (2 P-1 repeats)							
34	lef-8	36,618 ← 39,353	911 (105,338)		splt38 (918)	90 (832–921)	sf113 (881)	62 (569–917)	se112 (906)	62 (575–931)	ac50 (876)	58 (536–917)
35	bjdp	39,352 → 40,263	303 (34,813)		splt39 (300)	91 (276–301)	sf112 (397)	25 (63–248)	se111 (415)	25 (61–245)	ac51 (318)	27 (26–95)
36		40,286 → 40,855	189 (21,846)		splt40 (189)	88 (167–189)						
37		40,877 → 41,071	64 (7136)	L	splt41 (64)	62 (40–64)						
38	chitA	41,086 ← 42,885	599 (66,895)	L	splt42 (564)	94 (529–562)	sf22 (564)	57 (314–555)	se19 (572)	55 (304–555)	ac126 (551)	59 (326–548)
hr4	(5P-I repeats)	42,837...43,091			hr5 (2 P-1 repeats)							
39		43,305 → 43,907	200 (24,042)	EC	splt43 (204)	64 (130–203)						
40		44,073 ← 44,711	212 (25,313)		splt44 (208)	79 (168–212)	sf110 (179)	30 (59–194)	se109 (162)	23 (25–109)	ac52 (123)	26 (32–123)
41		44,783 → 45,229	148 (17,221)	L	splt45 (137)	85 (126–148)	sf109 (137)	47 (69–148)	se108 (137)	48 (70–147)	ac53 (139)	47 (58–124)
42		45,265 ← 46,533	422 (47,181)	L	splt46 (422)	79 (349–444)	sf108 (299)	33 (66–203)	se107 (344)	27 (61–222)		
43		46,538 ← 46,765	75	L	splt47 (75)	95 (71–75)	sf107 (67)	38 (25–66)				

Table 1 (*Continued*)

ORF or feature	Name[a]	Position[b]	# of aa (size, Da)	Promoter motifs[c]	SpltMNPV ORF (aa) or hr	SpltMNPV % ID (range)	SfMNPV ORF (aa)	SfMNPV % ID (range)	SeMNPV ORF (aa)	SeMNPV % ID (range)	AcMNPV ORF (aa)	AcMNPV % ID (range)
44	lef-10	46,725 → 46,973	82 (9025)	EC, L	splt48 (84)	86 (72–84)	sf106 (77)	49 (40–82)	se106 (77)	48 (39–82)	ac53a (78)	39 (28–72)
45	vp1054	46,813 → 47,850	345 (40,341)	e	splt49 (352)	91 (307–337)	sf105 (336)	46 (156–337)	se105 (346)	45 (157–348)	ac54 (365)	38 (135–360)
46		47,979 → 48,194	71 (8337)		splt50 (71)	80 (57–71)	sf104 (114)	38 (26–69)	se104 (67)	42 (32–76)	ac55 (73)	40 (23–58)
47		48,478 → 48,978	166 (19,688)	EC, L	splt51 (173)	93 (154–166)	sf102 (161)	37 (49–134)	se102 (178)	35 (47–134)	ac57 (161)	34 (54–160)
48		49,032 ← 49,601	189 (20,797)	L	splt52 (174)	70 (136–193)	sf101 (192)	59 (41–69)	se101 (199)	60 (38–63)	ac58 + ac59 (172)	
49		49,618 ← 49,866	82 (9469)	L	splt53 (82)	86 (57–66)	sf100 (88)	53 (35–66)	se100 (89)	59 (40–68)	ac60 (87)	43 (26–60)
50	cathepsin	49,913 → 50,923	336 (37,993)	e, L	splt54 (337)	93 (313–337)	sf20 (339)	52 (164–318)	se16 (337)	50 (163–326)	ac127 (323)	45 (151–334)
51	p49	50,972 ← 52,312	446 (51,346)		splt55 (439)	84 (375–446)					ac135 (299)	30 (68–228)
52	fp25k	52,420 ← 53,013	197 (23,009)	e, L	splt57 (197)	100 (197–197)	sf98 (195)	72 (130–181)	se98 (195)	72 (131–182)	ac61 (214)	60 (110–183)
53	lef-9	53,171 ← 54,667	498 (57,431)		splt59 (498)	96 (479–498)	sf97 (498)	69 (342–494)	se97 (497)	70 (46–491)	ac62 (516)	65 (316–489)
hr5	(8 P-I repeats)	54,677…55,304			hr6 (3 P-I and 20 P-II repeats)							
54		55,352 → 55,648	98 (10,569)									
55		55,681 → 55,989	87 (10,122)		splt5 (67)	56 (55–98)						
hr6	(3 P-I and 10 P-II-like repeats)	56,009…57,005			hr7 (2 P-I repeats)							
56		57,057 → 58,097	346 (40,542)		splt62 (367)	57 (212–372)						
57	rr2b	58,250 → 59,254	334 (38,740)		splt63 (333)	90 (302–335)	sf41 (384)	23 (14–61)	se45 (280)	53 (168–320)		
58	iap3/2	59,335 ← 59,742	135 (15,152)	L	splt64 (136)	73 (102–139)	sf87 (505)	30 (41–135)	se88 (317)	46 (26–57)	ac71 (249)	42 (25–59)
59		59,675 ← 60,616	313 (35,381)		splt65 (313)	85 (263–611)	sf88 (266)	42 (116–276)	se89 (299)	40 (115–285)	ac69 (262)	41 (111–270)
60		60,558 ← 60,974	138 (16,144)		splt66 (132)	91 (123–135)	sf89 (124)	58 (63–109)	se90 (133)	58 (63–109)	ac68 (192)	45 (49–109)
61	lef-3	60,979 → 62,028	349 (40,826)	e	splt67 (357)	89 (317–357)	sf90 (387)	29 (9106–367)	se91 (422)	29 (109–376)	ac67 (385)	27 (102–372)
62		62,263 ← 64,665	800 (92,439)		splt68 (790)	88 (709–807)	sf91 (712)	29 (81–277)	se92 (704)	31 (88–281)	ac66 (808)	37 (33–90)
63	dnapol	64,667 → 67,729	1020 (117,398	EC	splt69 (1022	90 (924–1027)	sf92 (1041)	54 (527–967)	se93 (1063)	54 (542–1005)	ac65 (984)	44 (431–979)
64		67,683 ← 67,871	62 (7756)									
hr7	(1 P-I and 3 P-II-like repeats)	67,916…68,117			hr8 (4 P-I repeats)							
65		68,224 → 69,366	380 (40,826)		splt70 (380)	81 (308–380)						
66		69,444 ← 70,067	207 (23,505)	L	splt71 (208)	77 (163–213)					ac74 (265)	22 (18–82)
67		70,139 ← 70,522	127 (14,621)	L	splt72 (127)	97 (123–127)	sf93 (128)	36 (45–126)	se94 (129)	35 (44–126)	ac75 (133)	23 (29–125)
68		70,545 ← 70,799	84 (9747)	L	splt73 (84)	99 (83–84)	sf73 (85)	59 (50–85)	se95 (85)	55 (47–85)	ac76 (84)	53 (19–36)
69	vlf-1	70,864 ← 72,015	383 (44,642)	L	splt74 (384)	97 (331–342)	sf84 (367)	66 (220–334)	se82 (372)	66 (221–334)	ac77 (379)	71 (239–338)
70		72,036 ← 72,398	120 (12,984)	e, L	splt75 (120)	73 (91–124)	sf83 (115)	34 (36–106)	se81 (127)	31 (38–121)	ac78 (109)	35 (37–105)
71	gp41	72,395 → 73,375	326 (36,401)	e, L	splt76 (330)	99 (301–305)	sf82 (332)	58 (175–301)	se80 (331)	58 (179–306)	ac80 (409)	60 (186–308)
72		73,350 ← 74,063	237 (27,678)		splt77 (233)	89 (211–238)	sf81 (244)	52 (112–214)	se79 (240)	57 (110–193)	ac81 (233)	51 (98–194)
73	tlp-20	73,924 ← 74,505	193 (21,164)	L	splt78 (197)	76 (154–203)	sf80 (196)	46 (65–141)	se78 (196)	51 (57–111)	ac82 (180)	30 (60–203)
74	vp91	74,474 ← 77,044	856 (97,482)	L	splt79 (861)	85 (734–862)	sf79 (814)	35 (300–857)	se77 (813)	36 (311–860)	ac83 (847)	32 (289–890)
hr8	(6 P-I repeats)	77,112…77,422			hr9 (2 P-I repeats)							
75	cg30	77,534 → 78,313	259 (29,974)		splt80 (250)	75 (193–257)	sf78 (458)	32 (22–69)	se76 (461)	31 (28–90)	ac88 (264)	25 (56–228)
76	vp39	78,342 ← 79,250	302 (33,935)	EC, L	splt81 (302)	98 (296–302)	sf77 (3280)	42 (124–297)	se75 (326)	44 (130–297)	ac89 (347)	37 (114–309)
77	lef-4	79,252 → 80,721	489 (56,305)		splt82 (475)	86 (420–489)	sf76 (459)	46 (227–497)	se74 (466)	48 (242–504)	ac90 (464)	39 (194–494)
78	p33	80,751 ← 81,518	255 (30,662)	e	splt83 (255)	97 (248–255)	sf75 (251)	52 (132–254)	se73 (252)	52 (133–255)	ac92 (259)	44 (116–262)
79		81,517 ← 82,050	177 (20,677)	EC	splt84 (182)	92 (166–182)	sf74 (159)	51 (79–156)	se72 (157)	52 (79–151)	ac93 (161)	48 (76–160)
80	odv-e25	82,047 → 82,727	226 (24,842)	E, L	splt85 (227)	95 (215–227)	sf73 (216)	62 (140–226)	se71 (216)	62 (140–226)	ac94 (228)	41 (87–214)
81	DNA helicase	82,832 ← 86,587	1251 (146,688)	L	splt86 (1235)	93 (1161–1254)	sf72 (1228)	40 (523–1228)	se70 (1222)	41 (531–1286)	ac95 (1221)	37 (474–1283)
82		86,556 → 87,071	171 (19,394)	L	splt87 (170)	96 (165–171)	sf71 (168)	51 (86–170)	se69 (170)	52 (88–169)	ac96 (173)	44 (72–162)
83	38k	87,079 ← 87,996	305 (36,252)	EC	splt88 (304)	94 (288–305)	sf67 (300)	53 (160–302)	se67 (300)	51 (151–298)	ac98 (320)	43 (134–312)
84	lef-5	87,892 → 88,785	297 (34,478)		splt89 (302)	84 (256–305)	sf66 (276)	52 (147–281)	se66 (279)	53 (149–282)	ac99 (265)	47 (119–254)
85	p6.9	88,806 ← 89,069	87 (10,175)	L	splt90 (84)	No similarity						
86	p40	89,130 ← 90,233	367 (41,649)	EC, L	splt91 (363)	95 (348–367)	sf64 (382)	44 (170–383)	se64 (388)	44 (172–389)	ac101 (361)	39 (143–366)

Table 1 (*Continued*)

ORF or feature	Name[a]	Position[b]	# of aa (size, Da)	Promoter motifs[c]	SpltMNPV ORF (aa) or *hr*	SpltMNPV % ID (range)	SfMNPV ORF (aa)	SfMNPV % ID (range)	SeMNPV ORF (aa)	SeMNPV % ID (range)	AcMNPV ORF (aa)	AcMNPV % ID (range)
					Comparison with other NPVs							
hr9	(8 P-I repeats)	90,305...90,732			*hr10* (4 P-I repeats)							
87	*p12*	90,770 ← 91,138	122 (13,569)	L	*splt92* (121)	83 (102–123)	*sf63* (101)	33 (31–95)	*se63* (106)	33 (27–83)	*ac102* (122)	30 (21–70)
88	*p45*	91,135 ← 92,262	375 (44,326)	L	*splt93* (373)	96 (357–373)	*sf62* (376)	59 (218–367)	*se62* (375)	59 (218–368)	*ac103* (387)	48 (184–382)
89	*vp80*	92,282 → 94,228	648 (73,807)		*splt94* (644)	85 (559–656)	*sf61* (574)	33 (66–199)	*se61* (556)	34 (56–165)	*ac104* (691)	29 (52–177)
90		94,225 ← 94,392	55 (6698)		*splt95* (55)	98 (54–55)	*sf60* (59)	56 (20–36)	*se60* (59)	44 (23–52)	*ac110* (56)	47 (14–30)
91	*odv-ec43*	94,421 → 95,506	361 (41,662)	e, L	*splt96* (361)	99 (357–361)	*sf59* (356)	48 (175–361)	*se59* (356)	48 (173–361)	*ac109* (390)	44 (174–394)
92		95,599 → 95,937	112 (12,971)	e	*splt97* (112)	92 (103–112)	*sf58* (114)	51 (39–77)	*se58* (114)	42 (35–83)	*ac108* (105)	43 (30–70)
93	*odv-e66*	95,927 ← 98,074	715 (80,801)	L	*splt98* (692)	93 (633–678)	*sf57* (685)	47 (306–647)	*se57* (723)	39 (266–676)	*ac46* (704)	35 (215–608)
94	*p13*	98,017 ← 98,874	285 (33,435)	L	*splt99* (289)	91 (262–289)	*sf56* (277)	56 (153–272)	*se56* (283)	57 (155–272)		
hr10	(8 P-I repeats)	98,878...99,348			*hr11* (4 P-I repeats)							
95		99,458 ← 99,982	174 (20,618)	EC	*splt127* (172)	58 (87–149)						
96		100,441 → 101,409	322 (37,138)	EC	*splt100* (321)	76 (246–322)	*sf53* (368)	33 (52–159)	*se54* (364)	32 (71–222)	*ac33* (182)	35 (13–37)
97		10,1451 ← 102,149	232 (26,766)	e, L	*splt101* (236)	86 (206–240)	*sf52* (217)	53 (122–231)	*se53* (222)	54 (127–237)	*ac106+ ac107* (243)	
98		102,169 ← 103,578	469 (52,661)	L	*splt102* (457)	80 (380–474)	*sf51* (517)	24 (49–208)	*se52* (529)	12 (26–208)		
99		103,593 ← 104,126	177 (20,618		*splt103* (179)	89 (159–179)						
100		104,211 ← 104,408	65 (7392)	e	*splt104* (66)	71 (47–66)						
101		104,495 → 105,790	431 (48,353)		*splt105* (418)	89 (372–420)						
hr11	(7 P-I and 24 P-II-like repeats)	104,511...107,449			*hr12* (9 P-I and 8 P-II repeats)							
102		107,481 ← 108,530	349 (40,891)		*splt106* (267)	59 (156–265)	*sf23* (205)	28 (38–134)				
103	*pif-3*	108,559 → 109,161	200 (22,764)	L	*splt107* (199)	92 (184–199)	*sf49* (209)	49 (98–199)	*se50* (214)	55 (95–174)	*ac115* (204)	49 (81–165)
104		109,276 → 109,512	78 (8870)		*splt108* (117)	85 (66–78)						
105	*alk-exo*	109,541 ← 110,788	415 (47,794)	L	*splt109* (408)	82 (337–411)	*sf39* (305)	41 (168–410)	*se41* (413)	44 (172–395)	*ac133* (419)	37 (157–424)
106		110,884 ← 111,843	319 (47,794)	e	*splt110* (377)	66 (207–316)						
107		111,873 ← 112,064	63 (7342)		*splt110* (377)							
108		112,210 ← 112,605	131 (14,852)	EC, L	*splt112* (127)	80 (105–131)	*sf40* (112)	29 (23–80)	*se42* (81)	29 (21–72)	*ac19* (108)	No similarity
109		112,607 → 113,839	410 (47,544)	EC	*splt113* (401)	84 (345–410)	*sf41* (384)	35 (143–412)	*se43* (280)	33 (135–411)	*ac18* (353)	22 (91–419)
110	*lef-2*	113,893 ← 114,669	258 (29,705)	e	*splt114* (254)	90 (189–209)	*sf16* (213)	37 (78–213)	*se12* (209)	38 (77–204)	*ac6* (210)	35 (76–215)
111		114,509 ← 114,931	140 (15,871)	e, L	*splt115* (114)	78 (94–121)	*sf15* (111)	27 (31–113)	*se11* (102)	35 (15–43)		
112	*p24capsid*	114,918 → 115,637	239 (26,776)	L	*splt116* (244)	91 (219–241)	*sf14* (237)	39 (89–227)	se10 (248)	39 (92–235)	*ac129* (198)	32 (71–221)
hr12	(7 P-I repeats)	115,638...116,055			*hr13* (3 P-I repeats)							
113		116,248 → 119,034	928 (106,194)		*splt118* (919)	83 (777–934)	*sf29* (876)	26 (188–724)	*se30* (886)	27 (231–863)		
114		119,051 → 119,782	243 (27,773)		*splt119* (181)	86 (158–184)	*sf28* (215)	30 (68–227)	*se29* (213)	31 (67–21)	*ac17* (164)	31 (34–111)
115	*bro-a*	119,836 ← 120,390	184 (21,746)	e	*splt120* (186)	88 (163–186)						
116	*egt*	120,636 ← 122,234	532 (60,890)	e	*splt121* (522)	87 (459–529)	*sf26* (207)	46 (179–369)	*se27* (523)	48 (250–523)	*ac15* (506)	41 (211–515)
117	*fgf*	122,363 → 123,094	243 (27,435)	e	*splt122* (246)	79 (196–249)	*sf37* (382)	28 (33–118)	*se38* (404)	29 (47–162)	*ac32* (181)	29 (40–136)
118		123,121 ← 123,354	77 (9233)	L	*splt123* (77)	91 (70–77)	*sf36* (85)	31 (11–35)	*se37* (80)	35 (18–51)	*ac120* (82)	No similarity
119	*pif-1*	123,359 ← 124,936	525 (59,572)	L	*splt124* (525)	83 (417–505)	*sf35* (529)	43 (211–489)	*se36* (526)	43 (217–506)	*ac119* (530)	40 (206–518)
hr13	(2 P-I repeats)	125,032...125,152			*hr14* (2 P-I repeats)							
120	*38.7k*	125,203 ← 126,240	345 (40,676)		*splt128* (342)	83 (285–343)	*sf17* (384)	32 (108–335)	*se13* (363)	32 (116–366)	*ac13* (327)	25 (75–306)
121	*lef-1*	126,227 ← 126,922	231 (40,430)		*splt129* (231)	93 (214–213)	*sf18* (239)	44 (97–221)	*se14* (216)	43 (95–222)	*ac14* (266)	42 (94–224)
122		126,903 ← 127,289	128 (14,473)	L	*splt130* (122)	82 (106–129)	*sf19* (140)	36 (28–78)	*se15* (154)	32 (37–114)		
123		127,286 ← 127,816	176 (21,056)	e	*splt131* (176)	90 (159–176)						
124	*calyx/pep*	127,826 ← 128,887	353 (39,060)	e, L	*splt132* (344)	96 (107–111)	*sf45* (332)	43 (151–354)	*se46* (335)	51 (51–100)	*ac131* (252)	29 (27–92)
125	*pkip*	128,976 → 129,515	179 (39,060)		*splt133* (205)	87 (157–180)	*sf31* (175)	32 (53–164)	*se32* (164)	28 (49–174)	*ac24* (169)	25 (28–114)
126	*arif-1*	129,549 ← 130,277	242 (27,280)		*splt134* (245)	89 (219–247)	*sf33* (298)	28 (48–173)	*se34* (281)	26 (63–247)	*ac20+ac21* (417)	
127	*pif-2*	130,305 → 131,570	421 (27,280)		*splt135* (425)	92 (371–404)	*sf34* (398)	59 (215–367)	*se35* (413)	58 (218–377)	*ac22* (382)	56 (209–374)

Table 1 (Continued)

ORF or feature	Name[a]	Position[b]	# of aa (size, Da)	Promoter motifs[c]	Comparison with other NPVs							
					SpltMNPV		SfMNPV		SeMNPV		AcMNPV	
					ORF (aa) or hr	% ID (range)	ORF (aa)	% ID (range)	ORF (aa)	% ID (range)	ORF (aa)	% ID (range)
hr14	(2 P-I and 6 P-II-like repeats)	131,598…132,033			hr15 (3 P-I and 26 P-II repeats)							
128		132,119 → 134,155	678 (76,644)	L	splt136 (682)	90 (614–684)	sf12 (702)	35 (241–686)	se8 (665)	36 (245–675)	ac23 (690)	27 (83–313)
129		134,189 ← 134,917	242 (76,644)	L	splt137 (231)	79 (150–190)						
130		135,022 → 135,798	258 (31,036)	EC	splt138 (259)	86 (223–259)						
131		135,885 → 136,295	136 (15,945)									
hr15	(6 P-II-like repeats)	136,511…136,711			hr17 (13 P-II repeats)							
132		136,765 ← 137,649	294 (35,386)	EC	splt140 (282)	47 (138–295)						

[a] P-I repeat: 5′-GAAAAGTCCGCCAGGTTCGATTCGAACTTGTCTGACTTTTC-3′ (underlined nucleotides differ in the majority sequence of SpltMNPV P-I repeats); P-II-like repeat: 5′-AACATGTT (N)$_{11-34}$.
[b] Arrows indicate 5′ → 3′ orientation of the ORFs.
[c] EC, early promoter motif; e, CGTGC motif within 210 bp of the initiation codon; L, late promoter.

lef-9 genes are available from GenBank under accession numbers JX454575 to JX454598.

3. Results and discussion

3.1. Basic features and ORF content of the SpliMNPV-AN1956 genome

Complete sequence determination of SpliMNPV-AN1956 revealed a 137,998 bp double-stranded DNA genome with a G+C content of 44.68%. *In silico Eco*RI digestion based on the sequence data agreed with the observed banding pattern following digestion of the genome by the same enzyme (data not shown). Extensive similarity at the nucleotide and protein level was noted between SpliMNPV and *Spodoptera litura* MNPV (SpltMNPV) (G2 strain; Pang et al., 2001), with a Martinez–Needleman–Wunsch alignment of the complete genome sequences yielding 85.5% sequence identity with 1403 gaps inserted to optimize the alignment. Although SpliMNPV contains 1344 fewer DNA base pairs by comparison, the two viruses proved to be nearly co-linear with one another across the bulk of their genomes. A schematic of the SpliMNPV genome indicating the location, relative size, and orientation of the ORFs and the homologous repeat region (*hrs*) is presented in Fig. 1.

Further analysis of the SpliMNPV sequence revealed 132 predicted ORFs transcribed from both strands (Table 1), including all of the 30 core genes found in all baculovirus genomes (McCarthy and Theilmann, 2008; van Oers and Vlak, 2007). The largest of these encoded DNA helicase (*spli81*) and DNA polymerase (*spli63*) and yielded proteins of 1251 and 1020 amino acid (aa) residues respectively, while *spli90* yielded the smallest of those surveyed at 55 residues. The identified SpliMNPV genes were compared with closely related viruses SpltMNPV (Pang et al., 2001), SfMNPV (Harrison et al., 2008) and *Spodoptera exigua* MNPV (IJkel et al., 1999) as well as AcMNPV (Ayres et al., 1994) (Table 1). SpltMNPV ORFs *splt6*, *splt9*, *splt22*, *splt25*, *splt56*, *splt58*, *splt60*, *splt61*, *splt111*, *splt117*, *splt125*, *splt126*, and *splt141* were not detected in SpliM-NPV; of these, only *splt125* (*bro-b*) has been described in other baculoviruses. SpliMNPV ORFs that bore no significant sequence similarity to those of the other four NPVs analyzed included ORFs *spli5*, *spli54*, *spli64*, and *spli131* (Table 1). Of these four ORFs, *spli54* exhibited 30% amino acid sequence identity to *Xestia c-nigrum* granulovirus (XcGV) ORF20 ($E = 5.9 \times 10^{-5}$), while the other three ORFs did not have BLAST matches with expected values lower than 1.8. XcGV *xc20* contains a chitin binding peritrophin-A domain found in other chitin binding proteins, in particular the peritrophic matrix proteins of insects and animal chitinases. This domain is also found in AcMNPV ORFs *ac145* and *ac150* and in homologous ORFs in other NPVs and GVs (Dall et al., 2001).

Conservation of amino acid sequences between SpliMNPV and SpltMNPV was an average of 85% identity across all genes; 53 SpliM-NPV ORFs bore at least 90% sequence identity to their SpltMNPV counterparts. SpliMNPV ORF132 proved the least conserved (47% identity) to *splt141*, its counterpart in SpltMNPV, and in only six other cases (ORFs *spli21*, *spli37*, *spli55*, *spli56*, *spli102*, and *spli132*) for which a SpliMNPV homolog was present in SpltMNPV was their shared similarity less than 60% (Table 1). Amino acid sequence identity with ORFs of SfMNPV and SeMNPV was lower in magnitude, with average values of 43.2% (range: 23–83%) and 43.9% (range 12–84%), respectively. AcMNPV was the most divergent, with an average sequence identity of 40.1% (range 22–85%). In each case, the polyhedrin gene was the most conserved among the analyzed viral sequences (Table 1).

Two pairs of homologous ORFs within the SpliMNPV and SpltM-NPV genome (*spli55*/*splt5* and *spli95*/*splt127*) were present in both viruses but the homologues of each pair were located in different
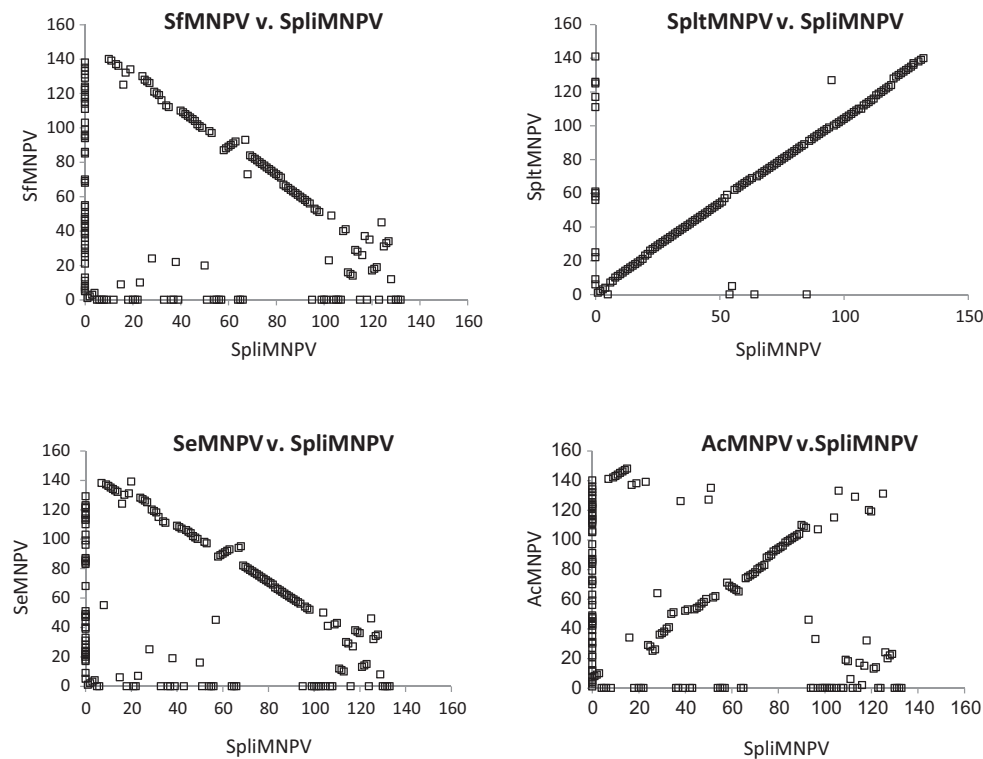
Fig. 2. Gene parity plot analysis of SpliMNPV (line) *versus* (clockwise order) SfMNPV, SpltMNPV, SeMNPV, and AcMNPV.

genomic regions. Interestingly, in the case of *spli55*, the corresponding SpltMNPV sequence was replaced with a unique gene, and in the case of *spli95*, the open reading frames corresponding to its preceding, upstream homologues in SpltMNPV (*splt125* and *splt126*, corresponding to SpliMNPV nt 123,341–123,185) proved absent. The proximity of homologous repeat regions (*hr*s) to these ORFs indicates a role for recombination in the different positions occupied by the homologues. In addition, a stop codon not present in *splt110* (376 aa) was detected in homologous ORF *spli107* at codon 63, which split the *splt110* homologue in SpliMNPV into two ORFs (*spli107* and *spli106*). Pang and colleagues (Pang et al., 2001) reported that SpltMNPV harbors a fused ubiquitin and gp37 gene (*splt32*) relative to SeMNPV and a novel baculovirus J-domain protein (*splt39*), thought to function in the conserved cellular stress response pathway (Pang et al., 2001). Homologues of these interesting genes were likewise detected in SpliMNPV at *spli28* (*ubi/gp37*) and *spli35* (*bjdp*) (Table 1).

Promoter motifs found in other baculovirus genomes (van Oers and Vlak, 2007) were also found in the SpliMNPV genome sequence (Table 1). Those ORFs unique to SpliMNPV did not possess any associated upstream promoter motif sequences.

Gene parity plot analysis revealed an inversion of the order of most of the ORFs of SpliMNPV compared to the ORFs of SfMNPV and SeMNPV, and an almost co-linear relationship of ORFs was observed for SpliMNPV and SpltMNPV (Fig. 2). Although many ORFs were not shared between SpliMNPV and AcMNPV, the order and directionality of their common ORFs were similar (Fig. 2).

3.2. Homologous repeat regions

The larger intergenic regions of baculovirus genomes often contain non-coding repetitive sequences. These homologous repeat regions (*hr*s) are elements that are thought to act as transcriptional enhancers and origins of DNA replication on the basis of transient expression and replication assays (Guarino and Summers,

1986; Hilton and Winstanley, 2007; Pearson et al., 1992). In alphabaculoviruses, the *hr*s often contain repeats of an imperfect palindromic sequence (Guarino et al., 1986; van Oers and Vlak, 2007). In AcMNPV, these palindromes are bound by the IE-1 transcriptional transactivator (Rodems and Friesen, 1995). The SpltMNPV genome was reported to possess 17 *hr*s containing one or both of two different repeated sequences: P-I, a 41-bp palindrome, and P-II, a 20-bp palindrome (Pang et al., 2001). Fifteen *hr*s containing similar repeat sequences were identified in the SpliMNPV-AN1956 genome. Fourteen of these *hr*s (*hr1–hr14*) contained copies of P-I, with the majority sequence differing at only two positions compared to the majority sequence of the SpltMNPV P-I repeat (Table 1). Repeats that resembled the P-II palindromes were also found, but sequence conservation after the first 8 bp was low among the repeats of different *hr*s and also when compared to the SpltMNPV P-II majority sequence. In some SpliMNPV *hr*s, the sizes of the presumptive P-II repeats also deviated significantly from 20 bp. These repeats were termed P-II-like repeats, with a majority sequence defined as 5′-AACATGTT (N)$_{11–34}$-3′ (Table 1). The SpliMNPV *hr*s occurred in similar places on the genome as the SpltMNPV *hr*s, but differed in the numbers of repeats present. In SpltMNPV, *hr2* and *hr3* are separated by ORF *splt25*. The SpliMNPV genome does not contain a homolog of *splt25*, and a single *hr* (*hr2*) containing both P-I and P-II-like repeats is found in this location. An *hr* corresponding to SpltMNPV *hr16* was not found in the SpliMNPV sequence. SpliMNPV *hr15* contains only P-II-like repeat sequences. A prior study (Faktor et al., 1997a) found that a 980-bp fragment of SpliMNPV-E15 containing *hr15* acted as an enhancer in transient expression assays, suggesting that the P-II-like repeats in *hr15* may play a role in viral gene expression.

Differences in ORF content and location between the SpliMNPV and SpltMNPV genomes correlated with the locations of SpliMNPV and SpltMNPV *hr*s, indicating a role for the *hr*s in the recombination events leading to ORF loss and rearrangement that has been suggested for group I alphabaculoviruses (de Jong et al.,

Table 2
Genetic variation within SpliMNPV-AN1956.

nt position	Consensus sequence	Variant sequence[a]	% reads[b]	Notes
801–812	aattttttcgtc	aaTttttt_g_c	11.6% (24/207)	Frameshift in spli2 (pp78/81)
3254–3265	(gt) x6	(gt) x7	14.3% (14/98)	Between spli3 and spli4
4856–4876	(atc) x7	(atc) x9	8.5% (12/142)	Expansion of Asp codon repeat region in spli4 (hoar)
27,171–27,184	tgtattggaaacag	tAtatt_aaacCg	10.4% (12/115)	hr2
35,715–35,718	cggc	cg**CCGGATTCG**gc	3.3% (5/150)	In-frame insertion of three codons into spli32 (p47)
41,090–41,105	aattccgaatgatgcg	aGttccgaatgatgAg	10.5% (14/133)	Silent substitutions in spli38 (chiA)
43,548–43,550	ttg	tCg	10.9% (14/128)	Gln to Arg substitution in spli39
43,909–43,924	(ttgg) x4	(ttgg) x6	15.1% (21/139)	Between spli39 and spli40
46,079–46,108	(ttagta) x5	(ttagta) x4	3.4% (4/116)	Contraction of Asn-Thrdicodon repeat region in spli42
47,135–47,140	gtttgg	gtttTgg	10.4% (19/183)	Frameshift in spli45 (vp1054)
47,149–47,154	ggcgaa	ggTCaa	8.2% (15/183)	Glu to Gln substitution in spli45 (vp1054)
51,084–51,086	cga	cAa	13.5% (31/229)	Silent substitution in spli51 (p49)
53,056–53,065	(ac) x5	(ac) x6	7.5% (13/174)	Between spli52 and spli53
59,531–59,548	(gat) x6	(gat) x4	1.5% (2/133)	Contraction of Ser codon repeat region in spli58 (iap3)
69,407–69,436	(ac) x15	(ac) x14	6.7% (9/134)	Between spli65 and spli66
69,436–69,438	cta	ctTa	6.1% (8/132)	Between spli65 and spli66
69,446–69,448	aca	acTa	33% (40/121)	Frameshift in spli66
69,452–69,454	tgc	tgAc	8.6% (10/116)	Frameshift in spli66
69,462–69,465	gatg	gaGtg	9.5% (11/116)	Frameshift in spli66
69,469–69,472	acta	acGta	8.7% (10/115)	Frameshift in spli66
69,508–69,511	acag	acTag	25.2% (27/107)	Frameshift in spli66
69,516–69,519	ggct	ggTct	22.1% (23/104)	Frameshift in spli66
69,528–69,531	gatt	gTAt	31.7% (33/104)	Ile to Tyr substitution in spli66
69,537–69,540	acac	acTac	10.5% (11/105)	Frameshift in spli66
69,543–69,546	cgat	cgTat	5.8% (6/104)	Frameshift in spli66
81,299–81,303	tcggt	tcTgt	8.7% (13/149)	Pro to Gln substitution in spli78 (p33)
81,545–81,562	(agc) x6	(agc) x7	12.2% (19/153)	Expansion of Gln codon repeat region in spli79
82,830–82,834	tttca	tActa	21.7% (15/69)	Silent substitution in spli81 (dnahel)
83,418–83,422	ttaac	ttCac	19.5% (33/169)	Silent substitution in spli81 (dnahel)
83,670–83,673	ctcg	ctAcg	7.1% (12/170)	Frameshift in spli81 (dnahel)
91,149–91,153	tatta	taCta	13.7% (23/168)	Asn to Ser substitution in spli88 (p45)
92,591–92,605	(ccg) x5	(ccg) x4	12.4% (19/153)	Contraction of Pro codon repeat region in spli89 (vp80)
93,017–93,021	cagcc	caAcc	9.7% (18/185)	Silent substitution in spli89 (vp80)
96,549–96,554	(gac) x2	(gac) x3	12.1% (19/157)	Expansion of Ser codon repeat region in spli93 (odv-e66)
97,864–97,875	(gct) x4	(gct) x5	12.6% (25/199)	Expansion of Gln codon repeat region in spli93 (odv-e66)
98,369–98,373	ggcgt	gCTgt	6.3% (11/175)	Silent substitution in spli94 (p13)
106,453–106,470	tggcaaagtcaagtgtcc	tAgcaTCTGcaagtgtTc	4.1% (3/73)	hr11
108,362–108,405	catcggtattgtccagatcggt attgtccagatccgtcttgtcc	c_____c	2.5% (3/118)	In-frame deletion of 14 codons from spli102
115,047–115,051	tgcaa	tgTaa	9.6% (17/177)	Silent substitution in spli112 (p24)
116,277–116,294	(ggc) x6	(ggc) x4	1.3% (2/156)	Contraction of Ala codon repeat region in spli113
118,680–118,683	aatc	aaGtc	7.0% (10/143)	Frameshift in spli113
118,805–118,809	ctagt	ctGgt	5.6% (6/107)	Ser to Gly substitution in spli113
126,191–126,195	gtttt	gtCtt	13.7% (22/161)	Silent substitution in spli120 (38.7k)
129,288–129,292	atagt	atGgt	7.9% (9/114)	Ile to Met substitution in spli125 (pkip)
134,652–134,679	aaatgatgcacacaat catttttaaca	aGatgatgcGcacaatcaa tttttaaTa	18.8% (30/160)	Silent substitutions and a Val to Ala substitution in spli129
135,451–135,455	ctcag	ctTag	13.6% (25/184)	Silent substitution in spli130

[a] Bold uppercase letters indicate insertions; underlined spaces indicate deletions, and italic uppercase letters indicate substitutions relative to the consensus sequence.
[b] Numbers of reads with the variation/total number of reads are indicated in parentheses.

2005). It is conceivable that recombination between the hrs corresponding to SpliMNPV hr1 and hr15 may be involved in the inversion of ORFs characterizing the divergence of SpliMNPV and SpltMNPV from SfMNPV and SeMNPV.

3.3. Genetic variation and relationships to other viruses

Genotypic variation can occur within a baculovirus isolate at high levels (Cory et al., 2005; Kamiya et al., 2003). The pyrosequencing technology used to sequence SpliMNPV-AN1956 resulted in an average coverage of 157X, a sampling depth that allows for a greater degree of categorization of the polymorphisms present in this NPV isolate than would be possible by conventional Sanger dideoxy methods. Table 2 presents a list of indels and substitutions either occurring at an incidence >5% or encompassing >3 nt. Many of the polymorphic sites involved variation in the numbers of di- or trinucleotide repeats, likely due to DNA polymerase slippage and/or uneven recombination within the region of the repeats (Debrauwere et al., 1997). Several of the polymorphisms

also were found in spli66, an ORF of unknown function also found in SpltMNPV and a small number of other NPVs.

Nucleotide sequences have been published from portions of other SpliMNPV-B isolates. Approximately 19 and 16.4 kbp of noncontiguous sequence data from SpliMNPV isolates M2 (Croizier and Croizier, 1994; Huang and Levin, 2001; Kikhno et al., 2002; Wolff et al., 1998) and E15 (Du et al., 1999; Faktor and Kamensky, 1997; Faktor et al., 1995, 1997a,b; Liu et al., 2003; van Strien et al., 1997), respectively, have been published and/or submitted to GenBank. A comparison of the SpliMNPV-AN1956 sequence with that of other isolates is shown in Table 3. Overall nucleotide sequence identity between SpliMNPV-AN1956 and other SpliMNPV-B isolates was high, indicating that SpliMNPV-AN1956 is a B-type isolate of SpliMNPV. However, the alignments were frequently characterized by substitutions and many gaps, some quite large. In three cases, substitutions and indels observed in the SpliMNPV-AN1956 isolate (Table 2) were also seen in sequences of other isolates (Table 3). Gaps in the alignments with other isolates sometimes occurred in ORFs, giving rise to frameshifts that truncated the ORF in the isolate
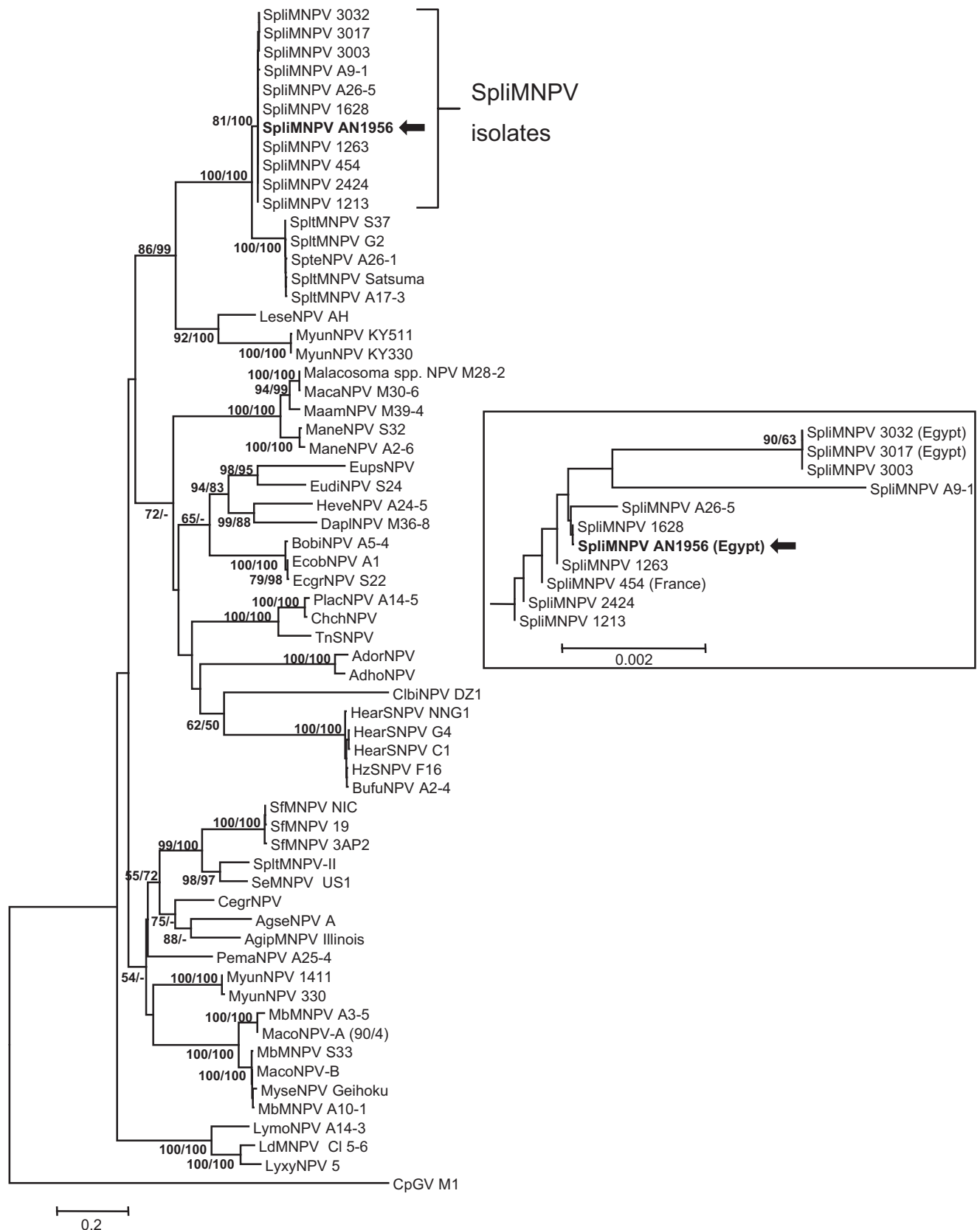
Fig. 3. Phylogenetic analysis of concatenated partial *polh*, *lef-8*, and *lef-9* nucleotide sequence alignments, showing bootstrap values for ME and MP trees at each node (ME/MP). Taxa include SpliMNPV isolates 454, 1213, 1628, 2424, 3003, 3017, and 3032 from a USDA insect virus collection sequenced for this study; a collection of group II NPVs reported by Lange et al. (2004) and Jehle et al. (2006) from *S. littoralis* (SpliMNPV A9-1 and A26-5), *S. litura* (SpltMNPV S37 and A17-3), *Spodoptera terricola* (SpteNPV A26-1), *Malacosoma* spp. (*Malacosoma* spp. NPV M28-2), *Malacosoma californicum* (MacaNPV M30-6), *Malacosoma americanum* (MaamNPV M39-4), *Malacosoma neustria* (ManeNPV S32 and A2-6), *Euproctis digramma* (EudiNPV S24), *Hemerocampa vetusta* (HeveNPV A24-5), *Dasychira plagiata* (DapiNPV M36-8), *Boamria bistortata* (BobiNPV A5-4), *Ectropis grisescens* (EcgrNPV S22), *Plusia acuta* (PlacNPV A14-5), *Busseola fusca* (BufuNPV A2-4), *Peridroma margaritosa* (PemaNPV A25-4), *Mamestra brassicae*

Table 3
Sequence comparison of SpliMNPV-AN1956 and other SpliMNPV isolates.

SpliMNPV isolate	Aligns with	% nucleotide identity/gaps	% amino acid identity with AN1956 ORF
SpliMNPV-M2 (Croizier and Croizier, 1994)	nt 137,729–137,998, 1–998	99.2%; 2 1-nt gaps, 1 2-nt gap, 1 3-nt gap, 1 4-nt gap, 2 5-nt gaps, 1 13-nt gap	*spli1* (*polh*): 99% *spli2* (*pp78/83*): 50% (residues 461–494, incomplete cds with a frameshift leading to a stop codon)
SpliMNPV-Az (Martins et al., 2005)	nt 1–747	99.7%; 1 3-nt gap	*spli1* (*polh*): 99.6%
SpliMNPV A9-1 (Jehle et al., 2006)	nt 189–699 (*polh*)	99.8% (*polh*)	*spli1* (*polh*): 100% (residues 64–233, incomplete cds)
	nt 37,485–38,204 (*lef-8*) nt 53,885–54,147 (*lef-9*)	99.4%, 1 6-nt gap (*lef-8*) 99.2% (*lef-9*)	*spli34* (*lef-8*): 99.2% (residues 384–623, incomplete cds) *spli53* (*lef-9*): 100% (residues 175–261, incomplete cds)
SpliMNPV A26-5 (Jehle et al., 2006)	nt 189–699 (*polh*)	100% (*polh*)	*spli1* (*polh*): 100% (residues 64–233, incomplete cds)
	nt 37,485–38,205 (*lef-8*) nt 53,885–54,147 (*lef-9*)	99.7%, 1 6-nt gap (*lef-8*) 100% (*lef-9*)	*spli34* (*lef-8*): 99.2% (residues 384–623, incomplete cds) *spli53* (*lef-9*): 100% (residues 175–261, incomplete cds)
Lab-SpliMNPV (Seufi, 2007)	nt 193–575	93%; 5 1-nt gaps, 1 3-nt gap, 1 48-nt gap	*spli1* (*polh*): 68% (residues 65–130, incomplete cds with a frameshift leading to a stop codon)
SpliMNPV (Egypt) (Seufi, 2008)	nt 241–645	98.5%	*spli1* (*polh*): 99.3% (residues 81–215, incomplete cds)
SpliMNPV-E15 (Faktor et al., 1997a)	nt 705–3704	99.4%, 7 1-nt gaps, 1 2-nt gap, 1 3-nt gap, 1 15-nt gap, 1 41-nt gap	*spli1* (*polh*): 100% (residues 235–248, incomplete cds) *spli2* (*pp78/83*): 90.9% (residues 24–356, truncated N- and C-termini) *spli3* (*pk-1*): 99.6% *spli4* (*hoar*): 100% identity (residues 751–792, incomplete cds)
SpliMNPV-E15 (Faktor et al., 1997b)	nt 18,092–20,253	99.4%; 3 1-nt gaps, 3 3-nt gaps	*spli15* (*odv-e56*): 94% (residues 353–370, incomplete cds) *spli16*: 100% *spli17* (*p10*): 100% *spli18*: 97.2% *spli19* (*p74*): 72% (residues 598–658, incomplete cds)
SpliMNPV, unspecified isolate (Faktor et al., unpublished sequence)	nt 19,994–22,191	99.3%; 4 1-nt gaps; 1 3-nt gap	*spli18*: 100% (residues 311–323, incomplete cds) *spli19* (*p74*): 95.9% *spli20* (*rr1*): 100% (residues 814–828, incomplete cds)
SpliMNPV-E15 (van Strien et al., 1997)	nt 21,983–24,815	99.4%; 6 1-nt gaps, 1 3-nt gap	*spli19* (*p74*): 100% (residues 1–20, incomplete cds) *spli20* (*rr1*): 97.9% (residues 49–828) *spli21*: 100% (residues 1–43, incomplete cds)
SpliMNPV-E15 (Faktor and Kamensky, 1997)	nt 36,422–39,498	99.6%; 3 1-nt gaps, 1 3-nt gap, 1 9-nt gap	*spli33* (*lef-12*): 100% (residues 190–198, incomplete cds) *spli34* (*lef-8*): 98.9% *spli35* (*bjdp*): 100% (residues 1–48, incomplete cds)
SpliMNPV-E15 (Du et al., 1999)	nt 50,867–52,487	99.6%; 1 1-nt gap	*spli50* (*cathepsin*): 100% (residues 319–336, incomplete cds) *spli51* (*p49*)[a]: 100% *spli52* (*fp25k*): 100% (residues 175–197, incomplete cds)
SpliMNPV-E15 (Liu et al., 2003)	nt 59,129–59,955	98.7%; 5 1-nt gaps, 1 3-nt gap, 1 6-nt gap	*spli57* (*rr2b*): 66% (residues 297–334, incomplete cds) *spli58* (*iap3/2*)[a]: 91.9% *spli59*: 100% (residues 221–313, incomplete cds)

(MbMNPV A3-5, S33, and A10-1), and *Lymantria monacha* (LymoNPV A14-3); SpltMNPV strains G2 (Pang et al., 2001) and Satsuma (Kouassi et al., 2009); *Mythimna* (*Leucania*) *separata* NPV strains AH (LeseNPV AH; Xiao and Qi, 2007) and Geihoku (MyseNPV Geihoku; Kouassi et al., 2009); *Mythimna* (*Pseudaletia*) *unipuncta* NPV (MyunNPV) isolates KY511, KY330, 330, and 1411 (Keathley et al., 2012); *Euproctis pseudoconspersa* NPV (EupsNPV; Tang et al., 2009); *Ecotropis obliqua* NPV (EcobNPV A1; Ma et al., 2007); *Chrysodeixis chalcites* NPV (ChchNPV; van Oers et al., 2005); *Trichoplusia ni* SNPV (TnSNPV; Willis et al., 2005); *Adoxophyes orana* NPV (AdorNPV; Hilton and Winstanley, 2008); *Adoxophyes honmai* NPV (AdhoNPV; Nakai et al., 2003); *Clanis bilineata* NPV strain DZ1 (ClbiNPV DZ1; Zhu et al., 2009); *Helicoverpa armigera* SNPV strains HearSNPV NNg1 (Ogembo et al., 2009), HearSNPV G4 (Chen et al., 2001), and HearSNPV C1 (Zhang et al., 2005); *Helicoverpa zea* SNPV strain F16 (HzSNPV F16; Chen et al., 2002); *S. frugiperda* MNPV strains SfMNPV NIC (Simon et al., 2011), SfMNPV 19 (Wolff et al., 2008), and SfMNPV 3AP2 (Harrison et al., 2008); *S. litura* MNPV strain SpltMNPV-II (GenBank ID: EU780426); *S. exigua* MNPV strain US1 (SeMNPV US1; IJkel et al., 1999); *Cerapteryx graminis* NPV (CegrNPV; Graham et al., 2011); *Agrotis segetum* NPV strain A (AgseNPV A; Jakubowska et al., 2006); *Agrotis ipsilon* MNPV strain Illinois (AgipMNPV Illinois; Harrison, 2009); *Mamestra configurata* NPV strains A 90/4 (MacoNPV-A (90/4); Li et al., 2005) and B (MacoNPV-B; Li et al., 2002); *Lymantria dispar* MNPV strain Cl 5–6 (LdMNPV Cl 5–6; Kuzio et al., 1999); and *Cydia pomonella* granulovirus strain M1 (CpGV M1; Luque et al., 2001). The location of the SpliMNPV isolates is indicated by a bracket, with an arrow denoting the position of SpliMNPV isolate AN1956. Inset: Higher resolution view of the SpliMNPV node.

Table 3 (*Continued*)

SpliMNPV isolate	Aligns with	% nucleotide identity/gaps	% amino acid identity with AN1956 ORF
SpliMNPV-M2 (Wolff et al., 1998)	nt 59,582–62,182	99.5%, 5 1-nt gaps, 1 2-nt gap	*spli58* (*iap3*): 100% (residues 1–53, incomplete cds)
			spli59: 100% (residues 1–109, with a frameshift leading to a stop codon) *spli60*: 100% *spli61* (*lef-3*): 99%
SpliMNPV-M2 (Huang and Levin, 2001)	nt 62,169–68,631	99%; 7 1-nt gaps, 2 2-nt gaps, 1 3-nt gap, 2 6-nt gaps, 1 62-nt gap	*spli62*: 100% (residues 1–109)
			spli63 (*dnapol*): 99.9% (residues 1–958) *spli64*: Not present due to 1-nt insertion in second codon *spli65*: 100% (residues 1–135, incomplete cds)
SpliMNPV-M2 (Kikhno et al., 2002)	nt 118,087–126,708	99.2%; 4 1-nt gaps, 1 2-nt gap, 2 3-nt gaps, 1 4-nt gap, 2 6-nt gaps, 1 15-nt gap, 1 25-nt gap	*spli113*: 98.1% (residues 614–928, incomplete cds)
			spli114: 97.1% *spli115* (*bro-a*): 98.4% *spli116* (*egt*): 99.8% (residues 18–532) *spli117* (*fgf*): 99.6% *spli118*: 100% *spli119* (*pif-1*): 99.8% *spli120* (*38.7k*)[a]: 99.1% *spli121* (*lef-1*): 94.3% (residues 73–231, incomplete cds)
SpliMNPV, unspecified isolate (Martin et al., unpublished sequence)	nt 119,118–124,325	99.8%; 2 2-nt gaps, 1 3-nt gap, 1 12-nt gap	*spli114*: 98.2% (residues 1–221, incomplete cds)
			spli115 (*bro-a*): 99.5% *spli116* (*egt*): 100% *spli117* (*fgf*): 99.6% *spli118*: 98.7% *spli119* (*pif-1*): 100% (residues 205–525, incomplete cds)
SpliMNPV-E15 (Faktor et al., 1995)	nt 120,455–122,381	99.2%, 5 1-nt gaps, 1 2-nt gap, 1 5-nt gap	*spli116* (*egt*): 99%
			spli117 (*fgf*): 100% (residues 1–6, incomplete cds)
SpliMNPV-E15 (Faktor et al., 1997a)	nt 135,837–136,777	96.8%, 10 1-nt gaps, 1 5-nt gap, 1 30-nt gap, 1 71-nt gap	Contains SpliMNPV*hr15*
			spli131: 75% (residues 1–85 with frameshifts and a premature stop codon) *spli132*: 100% (residues 292–294, incomplete cds)

[a] Contains substitution or indel found in SpliMNPV-AN1956 (Table 2).

being compared to SpliMNPV-AN1956. Notably, the ORF *spli2*, which encodes the PP78/83 capsid protein required for mobility of nucleocapsids in AcMNPV-infected cells (Ohkawa et al., 2010), is truncated in both SpliMNPV isolates M2 and E15 due to frameshifts. Much of the sequence data previously published for other SpliM-NPV isolates were determined using Sanger dideoxy sequencing with an ^{35}S-labeled dideoxynucleotide and autoradiography. It is possible that there are errors in these sequences that could account for the genetic differences reported in Table 3.

To characterize the relationship of SpliMNPV to other alphabaculoviruses and further explore the genetic variation occurring among SpliMNPV isolates, phylogenetic inference was carried out with concatenated alignments of partial *polh*, *lef-8*, and *lef-9* nucleotide sequence data from SpliMNPV-AN1956, other SpliM-NPV isolates in a USDA insect virus collection at Beltsville, MD, and other selected group II alphabaculoviruses, including NPVs from other *Spodoptera* species. The group II NPVs occurred in four major clades in the resulting tree (Fig. 3). SpliMNPV isolates occurred in a clade that included isolates of SpltMNPV and *Spodoptera terricola* NPV along with NPVs from the Oriental armyworm, *Mythimna* (*Leucania*) *separata*, and the true armyworm, *Mythimna* (*Pseudaletia*) *unipuncta* (Fig. 3). NPVs from *S. frugiperda*, *S. exigua* and a different NPV of *S. litura* (SpltMNPV-II) occurred in a separate clade that also included isolates from other noctuid cutworm and armyworm species (*Mythimna*, *Mamestra*, and *Agrotis*). The remaining group II NPVs in the tree occurred in two clades, one containing

Lymantria spp. NPVs, and the other containing a divergent group of viruses from *Adoxophyes*, *Malacasoma*, *Heliothis/Helicoverpa*, *Euproctis*, *Ecotropis*, and other species. In the group containing the SpliMNPV isolates, only a single well-supported clade occurred which contained two isolates from Egypt (3017 and 3032) and one from an unknown source (3003). SpliMNPV-AN1956 did not group with the other Egyptian isolates suggesting that the AN1956 isolate and the other Egyptian isolates may represent two different genotypes of SpliMNPV occurring within Egyptian populations of *S. littoralis*. Similar phylogentic groupings have previously been identified with Iranian isolates of the *Cydia pomonella* granulovirus which split between three different clades, two of which contained CpGV isolates from Georgia (the former Soviet Republic and Mexico (Eberle et al., 2009).

The close relationship of SpliMNPV and SpltMNPV isolates relative to each other in the Fig. 3 phylogeny mirrors the close relationship of the host species inferred from alignments of cytochrome-c oxidase subunit I sequences (Nagoshi et al., 2011). Pairwise genetic distances between the SpliMNPV and SpltMNPV isolates, estimated from concatenated alignments of *lef-8*, *lef-9*, and *polh* partial sequences with the Kimura-2-parameter model, ranged from 0.110 to 0.118 substitutions/site. Among the three individual loci, the *polh* sequences proved to be most conserved, with pairwise distances ranging from 0.070 to 0.082 substitutions/site between SpliMNPV and SpltMNPV isolates, and the least conserved locus was *lef-8*, with pairwise distances measuring from 0.148 to 0.163

Table 4
Dose-mortality (LC_{50}) and time-mortality (LT_{50}) response of neonate *S. frugiperda* infected with AcMNPV, SfMNPV, and SpliMNPV.

Virus	LC_{50}[a] (OBs/ml)	Fiducial limits		Slope	Median LT_{50}[a,b] (hr p.i.)	95% CL	% Mortality
		Lower limit	Upper limit				
SpliMNPV	1.02×10^6 b	7.82×10^5	1.35×10^6	1.9 ± 0.2	112.8 b	105.5–120.1	86.7
SfMNPV	9.39×10^5 b	7.14×10^5	1.24×10^6	2.2 ± 0.2	76.6 a	73.1–80.1	69.0
AcMNPV	1.69×10^6 a	1.29×10^6	2.24×10^6	2.0 ± 0.2	77.6 a	75.3–79.9	72.4

hr p.i.: hours post infection.
[a] Values with different letters are significantly different at $P < 0.05$.
[b] Median lethal time of insects infected was determined by the Kaplan–Meier estimator and reported with 95% confidence limits.

substitutions/site. According to the parameters for species demarcation proposed by Jehle et al. (2006), these distances indicate that SpliMNPV and SpltMNPV are different baculovirus species.

The divergence of *Spodoptera* spp. NPVs into two distinct groups can be only partially explained by the geographic distribution of the host species. *S. littoralis* and *S. litura* occur in Europe, Africa, the Middle East, and Asia, but not in the Americas, while *S. frugiperda* only occurs in the Americas. While SeMNPV groups with SfMNPV in phylogenetic trees, *S. exigua* originated in southeast Asia and is thought to have invaded North America as recently as 1876 (Greenberg et al., 2001). This observation, along with the occurrence of a second *S. litura* NPV (SpltMNPV-II) in the SfMNPV-SeMNPV clade, suggests that the divergence of SpltMNPV and SpliMNPV from SfMNPV and SeMNPV may have occurred prior to the appearance and current distribution of contemporary *Spodoptera* species.

3.4. Mortality

Bioassay analysis was performed to compare the insecticidal characteristics of SpliMNPV-AN1956, SfMNPV-3, and AcMNPV-C6. In neonatal *S. frugiperda* infected *per os* shortly following emergence, the LC_{50} for SpliMNPV-AN1956 was determined to be 1.02×10^6 OBs/mL, which was not significantly different ($P < 0.05$) from the LC_{50} of 9.39×10^5 OBs/mL determined for SfMNPV-3 (Table 4). This trend was in sharp contrast to the results of bioassays reported by Murillo and co-workers (Murillo et al., 2003), in which SpliMNPV-M2 killed 2nd-instar *S. frugiperda* larvae with an LC_{50} of 8.73×10^8 OBs/ml that was two orders of magnitude higher than the SfMNPV isolate Sf-2 LC_{50} of 4.31×10^6 OBs/ml. Both SfMNPV-3 and SpliMNPV-AN1956 LC_{50} values were markedly lower than the LC_{50} of AcMNPV-C6, which was 1.69×10^6 OBs/mL (Table 4). Median LT_{50} times for infected larvae were within 1 h of one another in the case of AcMNPV-C6 and SfMNPV-3 (76.6 h *versus* 77.6 h, respectively). The LT_{50} value for SpliMNPV-AN1956 extended to 112.8 h post-infection (Table 4), which was consistent with a prior study showing that SpliMNPV-M2-infected *S. frugiperda* larvae died with a significantly higher ST_{50} than larvae infected with SfMNPV Sf-2 (Murillo et al., 2003).

4. Conclusions

These data offer the first report of the complete genomic sequence determination of SpliMNPV, a baculovirus that has been employed in various parts of the Mediterranean and mid-East as a bio-control agent against the Egyptian cotton worm. The sequence proved very similar to that of SpltMNPV-G2, with the exception of a dozen gene deletions and two translocations that were likely caused by recombination after divergence of the SpliMNPV and SpltMNPV. SpliMNPV can infect and replicate in *S. litura*, and apparent isolates of SpliMNPV have been isolated from *S. litura* populations in Japan (Maeda et al., 1990; Takatsuka et al., 2003, 2007). However, genetic distances between isolates of SpliMNPV and SpltMNPV indicate that these viruses are not simply variants of the same baculovirus species.

In a series of bioassays developed in *per os* infected *S. frugiperda*, SpliMNPV-AN1956 proved to be more lethal than either AcMNPV-C6 or SfMNPV-3. In terms of killing speed, however, SpliMNPV-AN1956 lagged approximately 36 h behind the aforementioned baculoviruses. Further analysis of the differences between this virus and other related baculoviruses may help identify factors that contribute to the observed slower killing but overall increased mortality of SpliMNPV and such information, paired with genomic sequence data, may prove useful to investigators aiming to improve the efficacy of SpliMNPV against its natural host, or broaden viral host range of susceptible pest insects.

Competing interests

The authors declare that they have no competing interests.

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